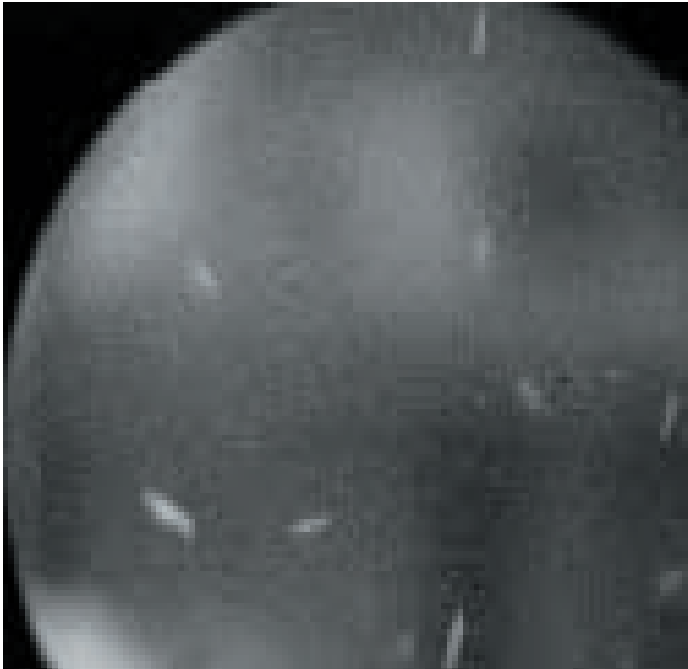


# Chapter 4

## Biological Physics





## Probing Gravitational Sensitivity in Biological Systems using Magnetic Body Forces

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At Brown University, we are developing the use of magnetic body forces as a means to simulate variable gravity body forces on biological systems. This tool promises new means to probe gravi-sensing and the gravi- response of biological systems. It also has the potential as a technique for screening future systems for space flight experiments.

## Applying B forces to learn about g forces

Gravity and magnetic  
body forces

Levitation of biomaterials  
(frog embryos)

Varying buoyancy  
(paramecia)

Controls



Alternatively, we have the shorter title *Applying B forces to learn about g forces*. Our work focusses on how gravity interacts with and affects life. This question is of practical and fundamental interest. The answers will impact our ability to perform deep space exploration. It is well known that the physiology of astronauts degrades, perhaps due to the lack of gravity, during space travel. In particular, they lose bone mass. The mechanism driving this loss is not known and has not found a complete remedy.

To address this practical issue, it is necessary to understand the fundamental issue of how gravity interacts with life. To study this interaction, we are developing a system and method to apply magnetic body forces to biological systems that can oppose or cancel the effects of the gravitational body force. We are testing this tunable body force environment on two model systems, frog embryos and paramecia, that exhibit well documented gravitational sensitivity at the few cell level. Both have been studied in low g environments and their responses are partially understood. I will describe our apparatus and how levitation of frog embryos may be the appropriate way to find out about their sensitivity and how varying the buoyancy of paramecia is probably appropriate for them. I will finish with a brief description of our

## Magnetism of Materials

2% ferro or para magnetic  
attracted to magnets:  
 $\text{Fe}$ ,  $\text{O}_2$

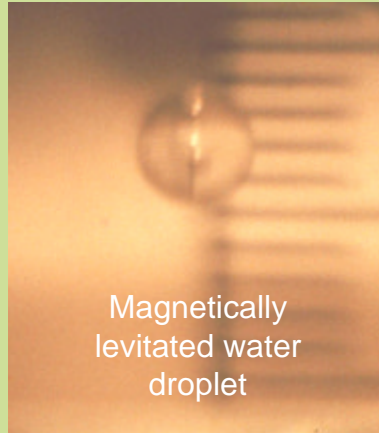
98% diamagnetic  
repelled by magnets  
water, protein



frog egg



paramecium



Magnetically  
levitated water  
droplet

The vast majority of the materials on earth are not magnetic in the common sense. That is, only 2% of materials are attracted to magnets. The other 98% of materials, including biological materials are diamagnetic materials and weakly repelled by magnets. In our work, we are using this repulsion to manipulate frog eggs and paramecium.



## Superconducting Solenoid Based Apparatus



11 mm dia. room temperature bore

$B_{\max} = 9.5 \text{ T}$

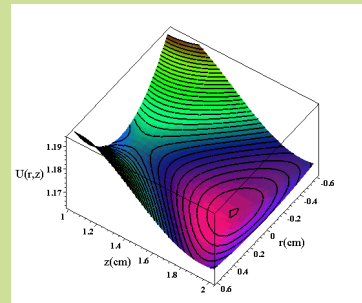
$(B \text{ dB/dz})_{\max} = 16 \text{ T}^2/\text{cm}$

5% force uniformity in  $(5 \text{ mm})^3$

Sample positioning to  $<1 \text{ mm}$

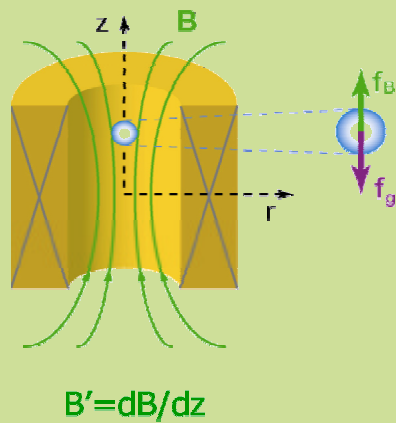
Borescope for in situ viewing

Advantages: long term  
experiments in a controlled lab  
environment



We are subjecting these biological specimens to the magnetic fields created by a superconducting solenoid based apparatus. This apparatus has a significant advantage over resistive magnet systems because once energized, they dissipate no energy. Consequently, magnetic fields can be applied to specimens for very extended periods.

## Magnetic Field Gradient Levitation



### Forces

$$f_B = m c_p B B' \quad f_g = -mg$$

### Levitates:

$$f_B = f_g$$

$$B B' = -\frac{g}{c_r} = -13.6 \frac{\text{Tesla}^2}{\text{cm}}$$

This magnet system is powerful enough that it can generate forces sufficient to levitate most common diamagnetic materials. Levitation of an object is achieved when the magnetic force is equal to and opposite its weight. The magnetic force depends on the magnetic susceptibility and the product of the magnetic field and the magnetic field gradient. Like the weight the magnetic force is proportional to the mass of the object and thus, is a body force, like gravity.

To give an example of the magnet strength necessary to levitate a common organic material, I point out that water levitates at -13.6 Tesla squared per centimeter. When levitated there are no gravitational stresses in the water (e.g. pressure gradients) because the magnetic force balances the gravity force molecule by molecule.

## Levitating Life

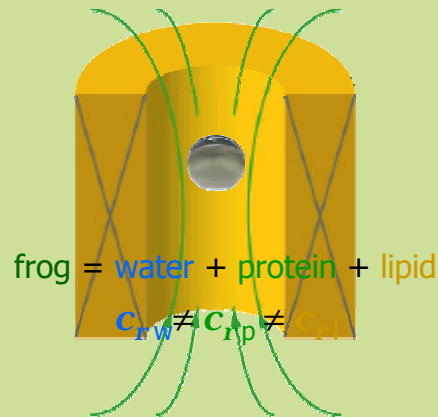
Levitation for:

$$BB' = -\frac{g}{\langle c_r \rangle}$$

$$\langle c_r \rangle = \frac{\sum_i m_i c_{r,i}}{\sum_i m_i}$$

Effective body force:

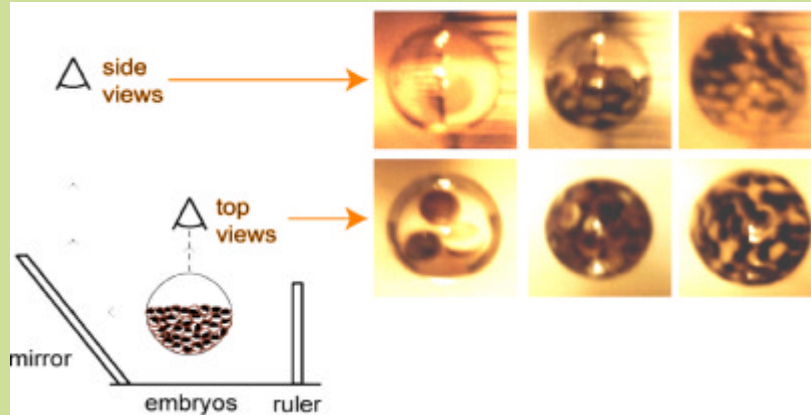
$$\frac{g_{eff,i}}{g} = 1 - \frac{c_{r,i}}{\langle c_r \rangle}$$



Want:  $c_{rw} \cong c_{rp} \cong c_{rl}$   
for reduced stresses

Levitation of a biological specimen, such as this frog egg, leads to a substantial reduction of the gravitational induced stresses on it, but not a complete reduction. Unlike a water droplet, a frog egg consists of a few different materials, which have different susceptibilities. The frog egg levitates at a field strength that depends on its average susceptibility. Fortunately, this average is close to the susceptibilities of its water, protein and lipid constituents. Thus, when levitated, the effective gravity on each of these constituents is reduced.

## Images of Levitating Embryos



JV, K. Lin, J. M. Denegre, and K. L. Mowry, *Biophys. J.* (1997)

We demonstrated that levitation reduces the gravitational stresses on frog eggs through a set of levitation experiments. This figure exhibits photos of three droplets of saline solution contain frog eggs. The left most frames show a droplet with only three eggs and the rightmost show a nearly full droplet. The field strength required to levitate these two were very similar, indicating that the susceptibility of the frog eggs is very similar to the susceptibility of the saline solution.

## MFGL Reduces Stresses



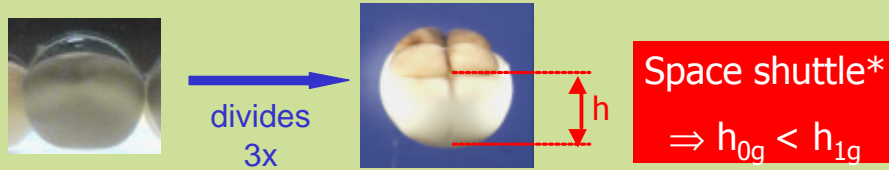
$$\langle \mathbf{c}_r \rangle_{egg} \cong 0.96 \mathbf{c}_{r,water}$$

Material	Volume %	$g_{eff}/g$
Pellet (proteins)	29%	0.075
Cytosolic (water)	66%	-0.02
Lipid	5%	0.06

⇒ Body forces reduced by more than  $\sim 10$ !

We measured the magnetic susceptibility of the constituents of the frog eggs by separating them through centrifugation and levitating them. We presumed that the centrifuged pellet was primarily protein, the cytosolic was primarily water and the supernatant was lipid. We concluded that the effective gravity acting on each of these constituents was reduced by more than a factor of 10 when an egg is levitated.

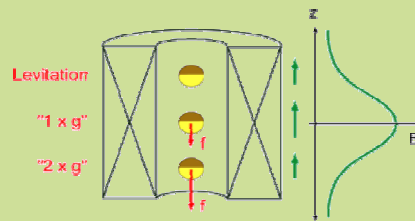
## Frog Embryo Gravitational Sensitivity



Goal:

Vary magnetic force  
timing, strength

Get same effect?



\* Black et al., PNAS ('94)

Frog eggs exhibit developmental features that depend on the presence or absence of gravity. In particular, the height of the first horizontal cell division in the eggs depends on gravity. We are investigating whether magnetic levitation alters the height in the same manner as 0 g does.

## Paramecium Caudatum



Paramecium Caudatum

by H. Horikami and M. Tsukii

### Characteristics

Single cell

Covered with cilia

Density,  $r_p = 1.04 \text{ g-cc}^{-1}$

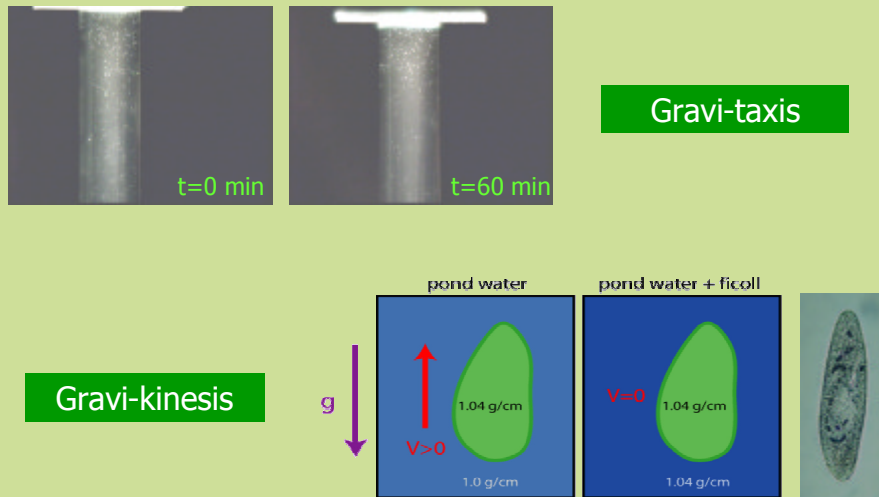
Lives at low Reynolds #

and, **gravi-sensitive....**

shown using drop towers,  
sounding rockets,  
centrifugation

Magnetic levitation is not necessarily the best way to apply magnetic forces to simulate low gravity for all biological systems. Many protists, such as paramecium, exhibit swimming behavior that depends on their buoyancy. The species that we have been studying, paramecium caudatum, are denser than their pond water and they move at low Reynold's number by beating the cilia that cover their bodies.

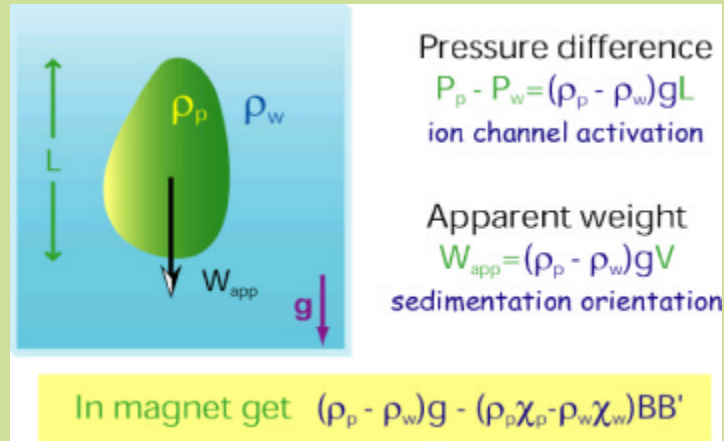
## Paramecia swim up



Remarkably, in the absence of cues that compete with gravity, Paramecium swim up, collecting at the top of any container in which they are placed. In addition to this gravitaxis, they also exhibit gravikinesis or the ability to adjust their swimming speed as they change their direction relative to gravity. In a neutral density medium, these gravi-responses disappear.



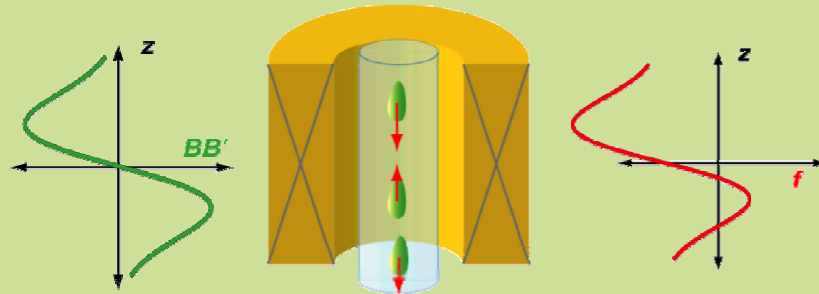
## Graviperception Models\*



\*there are others!

Various models for how paramecium sense gravity have been proposed. The most prominent models and experiments suggest that gravity couples to paramecium through their buoyancy. In one scenario, the buoyant weight of the cytoplasm stretches the lower parts of the cell membrane to activate ion channels, which in turn, pass ions that change the swimming behavior of the paramecium (Machemer and coworkers). In a second scenario, the paramecium are oriented by hydrodynamic stresses as they sediment and swim (Y. Mogami and coworkers). These mechanisms, one active and the second passive both depend on the buoyancy of the paramecium. As I describe in more detail in a moment, magnetic forces can be used to tune this buoyancy and thus, the coupling between paramecium and gravity.

## Varying Buoyancy in situ<sup>\*</sup>



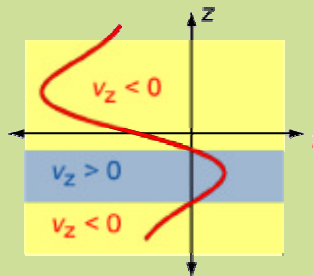
Significant Advantage:

Solution properties (e.g. chemistry, viscosity) remain constant

<sup>\*</sup> JM Valles and K. Guevorkian, J. Grav. Physiol. (2003)

Paramecium in a solution, in the bore of a solenoid experience a magnetic force that is proportional to the magnetic field-magnetic field gradient product (green curve) and a buoyant force. The sum appears qualitatively like the red curve. Notice that paramecium in the bottom region of the solenoid can experience a net downward force, paramecium a little higher in the solenoid experience a net force up and those in the top experience a net force down. Moving a vial containing paramecium into different positions in the solenoid effectively varies their buoyancy and does so without the need to alter the solution properties.

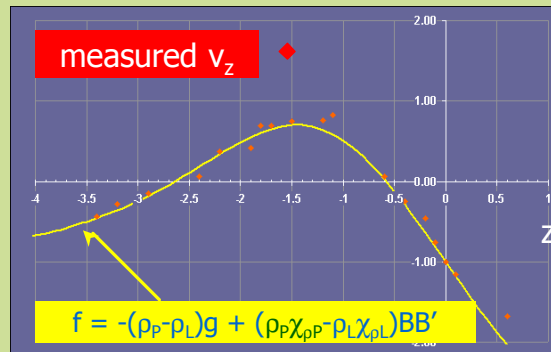
## Varying Paramecia Buoyancy proof of principle



$f_z \propto v_z$   
at low R for "fixed"  
paramecia

By carefully adjusting the properties, density and susceptibility, of the solution, we have been able to create a force curve similar to the one on the previous slide. The picture shows an image of a paramecium that has been "immobilized" (i.e. made unable to swim). It sediments upward when it is placed in the blue region and downward when placed in either of the yellow regions. The speed at which the paramecium moves is directly proportional to the net force acting on it because it moves at low Reynold's number.

## Measuring $\rho\chi_p$ of a single cell



Using

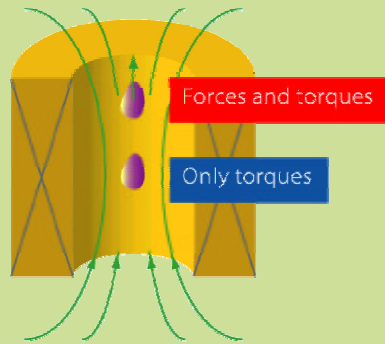
$$\begin{aligned}\rho_L &= 1.035 \text{ g/cc} \\ \rho_L \chi_{\rho L} &= -.729 \times 10^{-6} \\ \rho_P &= 1.04 \text{ g/cc}\end{aligned}$$

get

$$\begin{aligned}\rho_P \chi_{\rho P} &= -.723 \times 10^{-6} \\ \text{"the susceptibility of} \\ &\text{a single cell"}\end{aligned}$$

A series of measurements of the speed of the paramecium as a function of position in the solenoid provides a direct measure of the force curve. This curve depends on the susceptibility of the paramecium and the solution and their densities. A preliminary analysis of the speed as a function of position yields the magnetic susceptibility of a single paramecium!

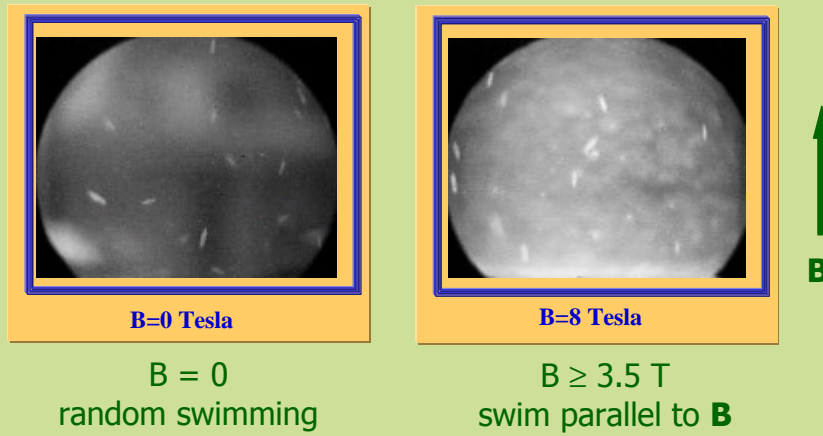
## Control Experiments



- **B** can exert forces
- **B** can exert torques
- Both can cause biological effects
- Use center of solenoid to control for torques

In applying this technique, we are only interested in the effects induced by the magnetic forces on the biological systems. However, magnetic fields, unlike gravity, can also exert torques on cells. Throughout our experiments we will control for “torque effects” by comparing cells placed in a magnetic force region with cells placed in the center of the solenoid where there is no magnetic force.

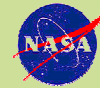
## P. Caudatum swimming in Magnetic Field



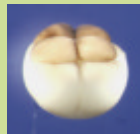
The effects of magnetic torques can be striking. In fact, we have found that paramecium swim nearly parallel to magnetic fields greater than about 3.5 Tesla. Given the fact that the magnetic field and gravity vector are parallel in our apparatus, this alignment will aid us in measuring changes in swimming speed with changes in force. It will make a larger portion of the population swim along the force direction and thus, provide us with better statistics. Also, our preliminary results suggest that magnetic fields do not alter the paramecium swimming speed.



## Applying B forces to learn about g forces



applies gravity  
sized forces



levitate



vary  
buoyancy

Goal: Probe gravitational sensitivity of biological  
systems with magnetic body forces.

We have begun investigations into the efficacy of applying magnetic body forces to study the effects of the body force of gravity on biological systems. Our apparatus makes it possible to levitate and vary the buoyancy of biological systems to simulate low gravity environments.

The interaction of positively charged ions with nucleic acid systems

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The successful development of extremely low background sample cells for x-ray scattering studies has enabled experiments designed to elucidate some of the fundamental physical interactions involved in macromolecular folding. Microfabrication techniques were used to fabricate low volume (50 microliter) sample cells with silicon nitride membranes (sub micron in thickness) as x-ray windows. Scientifically, these studies focus on the interaction of negatively charged nucleic acid systems, RNA and DNA, with their surrounding (positively charged) ion atmospheres. Understanding the structure of the ion atmosphere and its influence on the shape/conformation of the macromolecule will help reveal the underlying physical forces employed by nature in the self-assembly of these important molecules.